THE PEPTIN CONTENT OF SORGHUM GRAINS AND THEIR FRACTIONS

by

I-CHENG WANG

B. S., National Chekiang University, China, 1944

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Chemistry

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1956

TABLE OF CONTENTS

INTRODUCTION

The mixed calcium and magnesium salt of phytic acid, inositol hexaphosphoric acid, is commonly called phytin. It is widely distributed in the plant kingdom, occurring principally in the pericarp of seeds. The phosphoric acid combined with the inositol probably functions as a reserve phosphorus supply, to be used during germination prior to the absorption of phosphorus from the soil.

Phytic acid is capable of forming complexes with certain cations and has been used as a metal inactivating agent, or scavenger, in various industrial processes. The inositol portion of phytin is of biochemical interest, having been shown to be "bios 1" (Eastcott, 9), a substance necessary for normal reproduction of yeast cells. It is also a dietary essential which prevents the deposition of excessive amounts of fat in the liver.

The present industrial source of phytin is the steep liquor obtained during the processing of corn. As a part of an extensive research program concerned with sorghum grains, it was of interest to determine the content and distribution of phytin in these grains in order to evaluate their nutritive value and potential industrial utilization.

LITERATURE SURVEY

According to the structure of phytin, two ways are available for its estimation. One is to estimate the phytin phosphorus, the other is to determine the phytin inositol.

Phytin Phosphorus

Heubner and Stadler (18) introduced a volumetric method for the determination of phytin which was based on the fact that phytin is converted to insoluble iron phytate in dilute acid solution on the addition of ferric chloride. The phytin was titrated quantitatively with ferric chloride with ammonium thiocyanate as indicator, and the amount of phytin phosphorus was calculated from the amount of iron consumed by using the factor 1.19. They showed that inorganic phosphate and glycerol phosphate do not interfere with the titration under the conditions described. Their work was later reviewed and confirmed by Rather (27). However, Wrenshall and Dyer (29), by titrating the sodium phytate isolated from a soil extract, found that the phosphorus-iron ratio was between 1.11 and 1.28. Earley (8) studied the relationship of phosphorus and iron in iron phytate and found that the ratio was 0.833, which is almost the same as the theoretical ratio obtained from the formula $C_6H_6O_{24}P_6Fe_4 \cdot 3H_2O$ (2,3).

In addition to the variation in the phosphorus-iron ratio,

another difficulty associated with this method is that a white precipitate forms which obscures the end-point. Averill and King (4) suggested the use of more concentrated iron chloride. Harris and Mosher (16) modified the method by titrating until near the end-point, filtering to remove the whitish precipitate, and then completing the titration. Their work was modified further by Mione and Gandini (24), who detected the end-point by following the development of turbidity during titration with ferric chloride. Knowles and Watkin (19) and Lopez and Moreno (21) proposed sodium salicylate as an indicator because the violet end-point so obtained was easier to detect than the yellow-brown color obtained with ammonium thiocyanate.

In order to avoid the difficulties which are encountered in the ferric chloride method described above, McCance and Widdowson (22) estimated the phytin content of a number of foodstuffs by precipitating the phytin as iron phytate, boiling the precipitate with sodium hydroxide, filtering out the ferric hydroxide which formed, oxidizing the organic matter of the filtrate with concentrated sulfuric and nitric acids, and determining the phosphorus content of the digest by the molybdenum blue method. A similar technique was employed by Young (31). The method of McCance and Widdowson was employed by Pons et al. (26) for the determination of phytin in plant materials. Both Young and Pons reported that the precipitation of phytic acid by ferric ion is quantitative. However, in the determination of phytin in wort and beer, Essery (10) reported that application of heat to

facilitate the precipitation markedly increased co-precipitation of inorganic phosphate. He eliminated the inorganic phosphate by re-precipitating the phytin three times from a 1/6 N solution of hydrochloric acid by the addition of ferric chloride.

On the other hand, a slightly different method of determination was employed by Michel-Durand (23), who precipitated both phytin and inorganic phosphate with magnesium oxide. The precipitate so obtained was dissolved in aqueous 10 per cent CCl_3COOH, and the phytin was precipitated by adding calcium acetate.

Inositol

The most common chemical method of estimating inositol depends upon its oxidation by periodic acid and measurement of the amount of periodic acid consumed. The exact mechanism of the oxidation is not clear. After extensive studies on this problem, Fleury *et al.* (12,13) suggested that the inositol molecule is initially attacked at two points, with the formation of two moles of tartronic dialdehyde. The dialdehyde rapidly rearranges to hydroxypyruvic aldehyde, which then is cleaved to glycolic acid and formic acid.

$$C_6H_6(OH)_6 + 2HIO_4 \longrightarrow 2CHO\cdot CHOH\cdot CHO + 2H_2O + 2HIO_3$$

$$2CHO\cdot CHOH\cdot CHO \longrightarrow 2CH_2OH\cdot CO\cdot CHO$$

$$2CH_2OH\cdot CO\cdot CHO + 2HIO_4 + 2H_2O \longrightarrow 2CH_2OH\cdot COOH + 2HCOOH + 2HIO_3$$

$$2CH_2OH\cdot COOH + 4HIO_4 \longrightarrow 2HCOOH + 2CO_2 + 2H_2O + 4HIO_3$$

However, Fleury stated that the oxidation stops before the glycolic acid is oxidized completely. According to Fleury, the number of oxygen atoms consumed per molecule of inositol is in the range 6.2 to 6.7. He reported that variations in the relative proportions of inositol and periodic acid, in pH (1.2-6.0), and in temperature (0-50°C.) did not affect the extent of the oxidation. Fleury and Nocoulos (14) also estimated inositol in biological materials by measuring with a Warburg apparatus the carbon dioxide which is evolved during periodate oxidation. Platt and Glock (25) determined inositol by measuring the formic acid which is liberated during the oxidation.

Before the inositol can be determined, it first is necessary to hydrolyze the phytin. The hydrolysis usually is carried out in an acid medium, inasmuch as hydrolysis is not complete if the solution is alkaline (Fleury, 15). Beadle (6) estimated inositol with a biological method after refluxing the phytin in 18 per cent HCl for six hours, and he reported that only one-third of the calculated yield of inositol was obtained. Fleury (15) observed that the rate of hydrolysis of phytic acid varied with the pH, the maximum rate occurring at pH 3. Hoggen and Reith (17) hydrolyzed phytin preparations with 35 per cent H_2SO_4 for eight hours under reflux condition. A similar technique was employed by Bailly (5). However, Soligson, as quoted by Lindenfeld (20), used a 50 per cent H_2SO_4 solution at 160°C. for 12 hours, and obtained only 89 per cent hydrolysis.

Industrially, phytin is hydrolyzed in water solution under

pressures as high as 85 pounds per square inch for five or six hours. The best yield of inositol under these conditions was 12-13 per cent, as reported by Bartow and Walker (7). The theoretical yield from phytin should be 19.2 per cent.

EXPERIMENTAL

From the survey of the literature, it became apparent that the earlier methods of determining phytin by titrating with ferric chloride were inadequate, since there was considerable disagreement concerning the factor to be used for calculating the phosphorus equivalence of the ferric ion consumed. Apparently the phosphorus-iron ratio is affected by the conditions of precipitation. It is probable that some ferric ion is adsorbed by non-phytin organic matter which is present. It was concluded that a method involving precipitation of phytin by ferric ion and direct determination of the phosphorus content of the precipitate would be the most precise. Such a method would eliminate the complications which arise due to the variable consumption of ferric ion. It seemed plausible that measurement of the inositol content of the precipitate also would be a suitable means of determining phytin. A study was made of these two methods to determine their suitability for estimating the phytin content of sorghum grains.

Preparation of Sorghum Grain Samples

The dry milling process used in this study reduced the sorghum grain into five fractions: bran (outermost seed-coat layers), bran fines (remaining seed-coat layers), germ (embryo), grits (endosperm), and mill fines (endosperm admixed with germ and bran fragments).

The whole grain was cleaned to remove chaff and cracked grains. The grain was tempered by adding 5 per cent water. After standing for two hours, the grain was debranned by repeatedly passing it through a debranner until all the bran was removed. Usually 20 to 40 passes were required.

The bran fines were removed by sifting the sample on a 60-mesh screen. The bran-cracked grain mixture which remained on the screen was passed through an aspirator to remove the bran from the cracked grain.

The decorticated grain next was screened with a screen stack consisting of 9 and 20 mesh screens. The "overs" of the 9-mesh screen were passed through an impact mill. This process was repeated until all of the decorticated grain was fine enough to pass through the 9-mesh screen. The fraction which passed through the 20-mesh screen was called the "mill fines." The fraction retained by the 20-mesh screen was aspirated to remove any bran which might be present. This bran was added to the "debranner" bran. The material retained by the 20-mesh screen, consisting of germ and grit, was separated by means of differences

in density by using an air-gravity separator.

The whole grain and its coarse fractions, germ and grit, were ground in a Wiley mill, equipped with a 20-mesh screen. The other fractions were sufficiently fine that they required no further grinding.

Preparation of Solution of Calcium Phytate for Hydrolysis

Two grams of commercial calcium phytate (Nutritional Biochemicals Corporation) was dissolved in 50 ml. of 0.6 per cent HCl, and the solution was centrifuged to remove insoluble matter and was filtered through asbestos under suction. The solution was diluted with 0.6 per cent HCl to 1000 ml., and the total phosphorus and inorganic phosphorus contents were determined.

Extraction and Precipitation of Phytin

A suitable quantity of sample (for the determination of phytin phosphorus: 5 g. for whole grain and bran, 1 g. for germ, and 10 g. for mill fines; for the determination of inositol: half of the above quantities) was extracted with 50 ml. of 2 per cent HCl by soaking at room temperature, with swirling at 15 minute intervals, for two hours. The mixture was centrifuged to remove the solids, and the extract was further clarified by filtering it with suction through an asbestos pad.

To 10 ml. of the extract in a centrifuge tube were added

10 ml. of 0.3 per cent [illegible] solution and sufficient water to make the resulting solution approximately 0.6 per cent with respect to hydrochloric acid. Ferric chloride solution (0.002 g. of ferric ion per ml.) was added dropwise until a permanent brown color due to ferric thiocyanate was formed. The mixture was allowed to stand for a half-hour and then was centrifuged. The precipitate was washed twice with about 20 ml. of 0.6 per cent HCl, the wash solution being removed by centrifuging and decanting. The buff colored precipitate (iron phytate) so obtained was analyzed for phosphorus or inositol as described below.

Determination of Phytin Phosphorus

The iron phytate precipitate was washed into a 100 ml. beaker with water. A few drops of 6N HCl were added to dissolve the particles which adhered to the wall of the centrifuge tube, and the tube was rinsed with water. The solution was evaporated to dryness, 2 ml. of concentrated H_2SO_4 and 2 ml. of concentrated HNO_3 were added, and the mixture was heated to destroy the organic matter. Additional nitric acid was added three times to insure complete oxidation. A few drops of perchloric acid were added to expel the excess nitric acid. The last trace of nitric acid finally was expelled by adding 20 ml. of water and evaporating to a small volume. The residue was diluted to 250 ml., and 10 ml. of this solution were used for the colorimetric

determination of phosphorus by the A.O.A.C. method (1).

A 10 ml. aliquot was transferred to a 100 ml. volumetric flask, and three drops of a 0.2 per cent sodium alizarin sulfonate solution and exactly 5 ml. of 0.5 N NaOH were added. Then sulfuric acid (1 N) was added until the solution just became yellow. Exactly 10 ml. of 8 per cent sodium bisulfite solution was added, and the solution was heated in a boiling water bath for one hour. Ten ml. of diluted molybdenum blue reagent was added, and heating was continued for exactly 20 minutes. The solution was cooled quickly, made to volume, and the intensity of the blue color was measured with a Beckman spectrophotometer at 650 mμ.

Determination of Inositol

One ml. of 6N HCl was added to the iron phytate in the centrifuge tube, and the tube was heated in a boiling water bath for a few minutes to dissolve the phytate. Sufficient hot NaOH (0.5N) was added to precipitate the iron. The solution was filtered and the residue was washed with hot water several times. The filtrate containing sodium phytate was adjusted to pH 2.8 with dilute sulfuric acid, using a Beckman pH meter. The solution then was hydrolyzed at a pressure of 10 pounds per square inch for 15 hours.

Ten ml. of 0.02N HIO_4 were added to the hydrolysate. The oxidation was carried out at 30 ± 1°C. for 12 hours. The solution

then was buffered with 5 ml. of saturated $NaHCO_3$, and 5 ml. of a 0.1 N standard sodium arsenite solution were added. Two ml. of 10 per cent KI were added to serve as a catalyst. After five minutes the excess arsenite was titrated with standard 0.05 N iodine solution, using starch as an indicator. The reaction was standardized by oxidizing 1 ml. of pure inositol, and from the amount of periodic acid consumed it was possible to calculate the inositol content of the samples.

The number of oxygen atoms consumed for each molecule of inositol also can be calculated, according to the following equation:

$$C_6H_6(OH)_6 + 6HIO_4 \longrightarrow 6HCOOH + 6HIO_3$$

RESULTS AND DISCUSSION

Extraction

Reported methods for the quantitative extraction of phytin differ in acid concentration and time of extraction. To determine the best conditions for removing phytin from sorghum grain, extractions were made with 1 per cent and 2 per cent HCl for one, two, and three hours, as indicated in Table 1.

Maximum extraction was obtained in one hour with 2 per cent HCl. One per cent HCl was suitable only if the time of extraction was more than two hours. On the basis of these data, the extraction procedure selected for the remainder of this study

Table 1. Extraction of the Phytin of sorghum germ

Extraction time (hours)	Treatment	Phytin	
		1% HCl	2% HCl
		%	%
1	Swirling	—	1.73
	every 15 min.	—	1.69
2	Swirling every half-hour	1.49	1.66
		1.55	1.68
3	Constant shaking	1.60	1.69
		1.63	1.69

consisted of soaking the sample in 2 per cent HCl for one hour with frequent swirling. There was no advantage in using a shaker. It should be mentioned that when a shaker was used, the extracts were very difficult to filter.

Phytin Phosphorus

In determining phytin by measuring the phosphorus content of iron phytate, consideration must be given to (1) the completeness of isolation of phytin as iron phytate, and (2) the possible interference of iron. In the latter case, it was found that the iron exerted no interference on color formation with molybdenum blue reagent when an adequate quantity of sodium bisulfite was used (30). Two calibration curves were prepared, one with and one without iron being present. From Fig. 1 it will be seen that the two calibrations were identical.

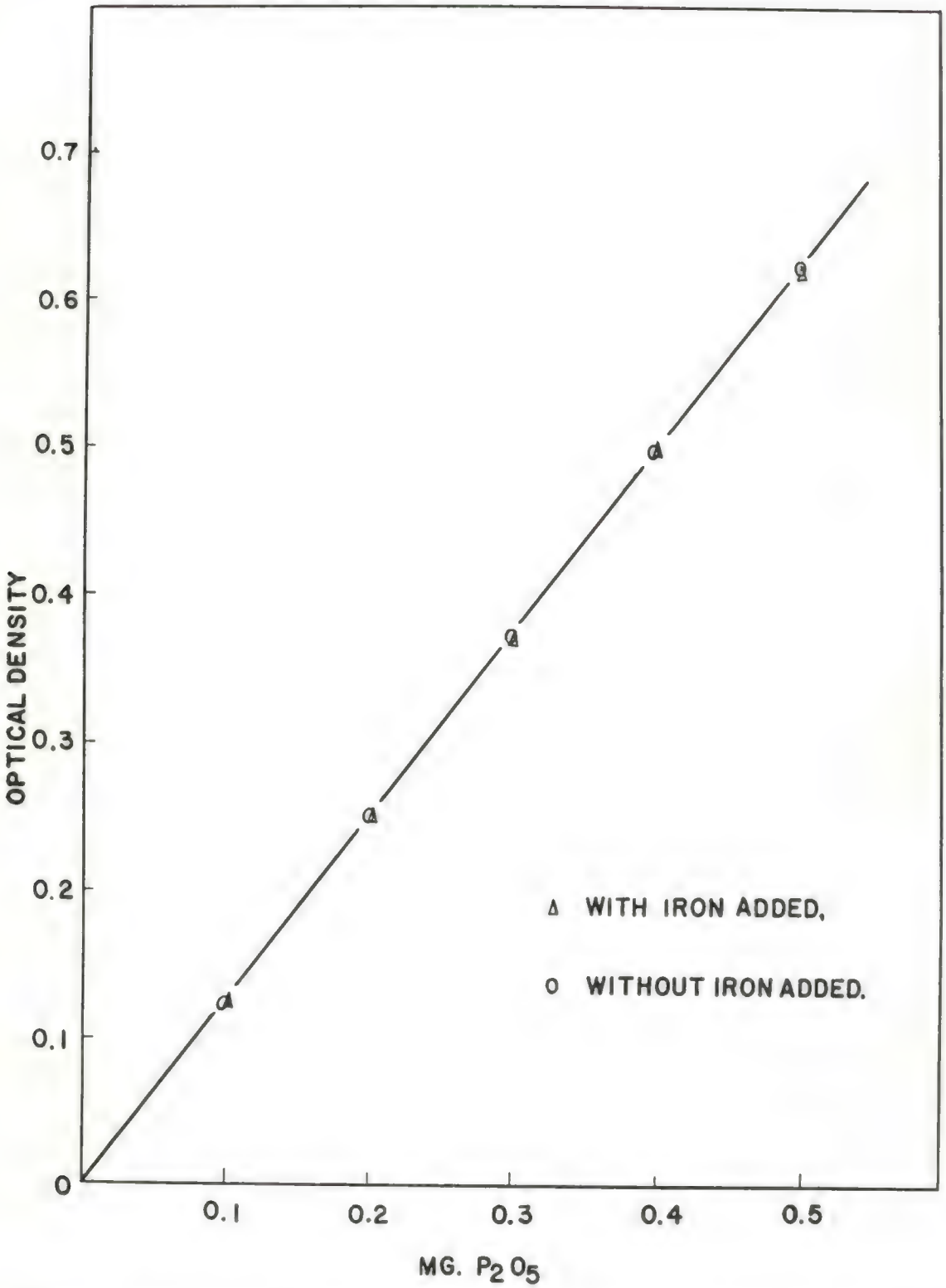

Fig. 1. Calibration curve obtained with standard KH_2PO_4 solution.

The isolation of phytin from the extract was the next point to be considered. Previously, the isolation had been done by adding ferric chloride and heating to promote coagulation of the colloidal iron phytate. It was thought that heating might cause coprecipitation of other phosphates or of oxidizable substances which would complicate the subsequent determination of inositol. Therefore, precipitation of iron phytate at room temperature was studied. Purified calcium phytate was analyzed for total phosphorus by the oxidation procedure. A sample of the calcium phytate then was subjected to the phytin phosphorus procedure. The results show (Table 2) that the precipitation of iron phytate at room temperature was quantitative.

Table 2. Recovery of Phytin phosphorus from calcium phytate

Treatment	: mg. Phosphorus per mg. Sample
Total Phosphorus of Calcium Phytate	0.16 0.15
Phytin Phosphorus by Ferric Chloride Precipitation	0.15 0.15

Phytin Inositol

In this study it was found, as Fleury has shown, that inositol utilized somewhat more than the theoretical quantity of oxygen for oxidation. The number of oxygen atoms consumed for each molecule of inositol varied from 6.2 to 6.6, which is in

agreement with the results of Neuy (Fig. 2). At 30°C. the oxidation was complete in twelve hours in water or slightly acidic medium (pH 5-6). In preliminary work it was found that when the oxidation was carried out in a medium at or below pH 1.4, low or erratic results were obtained.

A study of the hydrolytic conditions for the release of inositol from phytic acid of corn revealed that under certain conditions inositol is not released completely (Table 3). Thus, low values for both inositol and phosphorus were obtained when hydrolysis was carried out at 15 pounds per square inch five hours at pH 1.4, 2.3, and 3.9. However, when hydrolysis was conducted at pH 2.8 for 15 hours at 10 pounds of pressure per square inch, good recoveries were obtained. Under these conditions, 98 per cent of the phosphorus and 102 per cent of the inositol were liberated and measured. The inositol-phosphorus ratio was 0.977, which compares favorably with the theoretical ratio of 0.968 for inositol hexaphosphoric acid.

Phytic acid may not occur in nature exclusively as the hexaphosphoric acid ester. For example, the tetraphosphoric acid ester has been reported to be present in wheat bran (Winsowdelm, 28). Thus, the phytic acid of sorghum grain also might be something other than the hexaphosphate. To determine this, it was necessary to obtain the inositol-phosphorus ratio of highly purified phytic acid isolated from sorghum grain. Such a ratio was needed also for detecting possible precipitation of non-phytin phosphorus along with iron phytate during

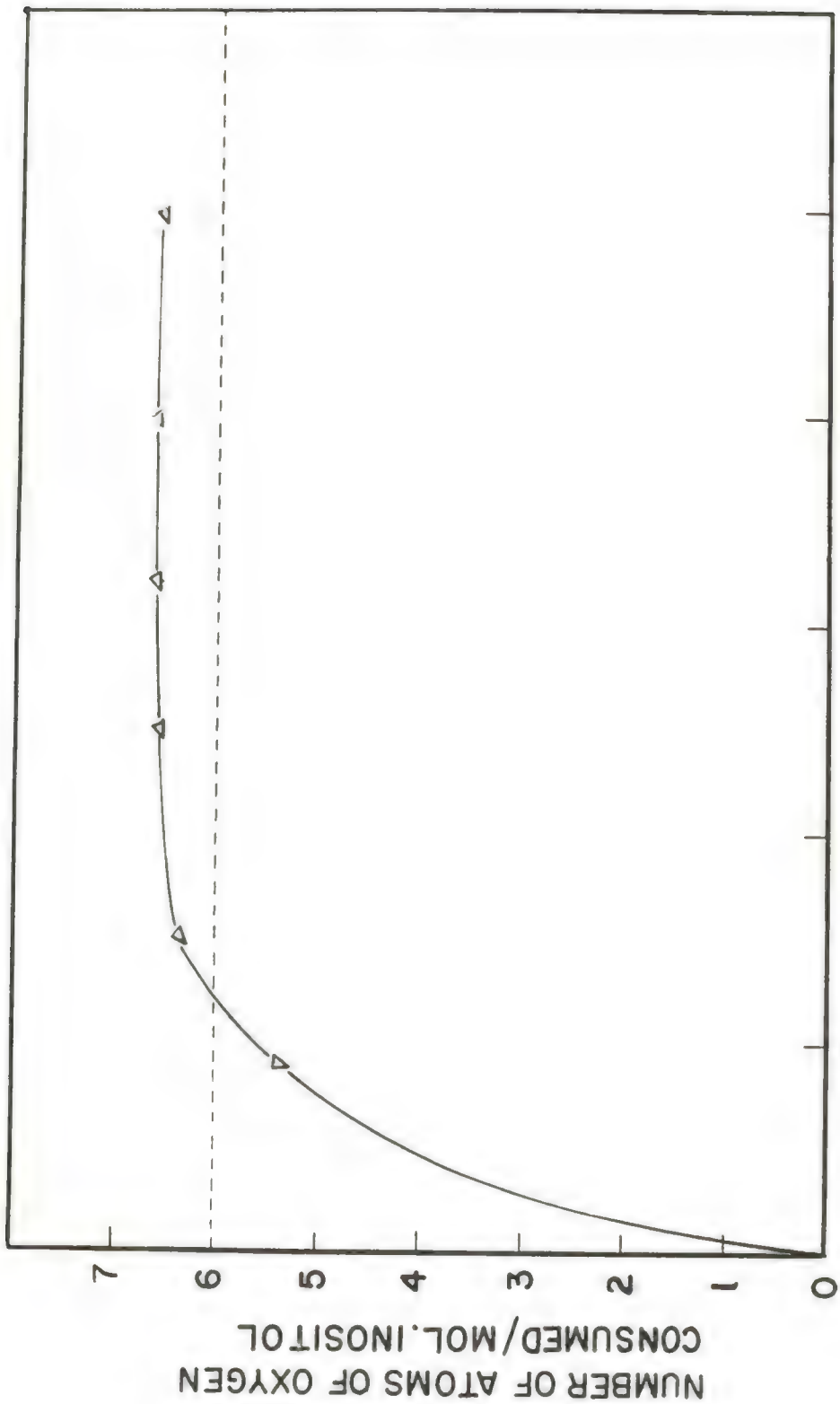

Fig. 2. Oxidation of Inositol by Periodic Acid at 30°C. (1 mg. inositol in 100 ml. water.)

Table 3. Hydrolysis of Calcium Phytate

Acidity	Pressure	Temperature	Time	Total Acid Soluble P	After Hydrolysis		Percent Hydrolysis based on P Liberation
					P	Inositol	
pH	P. S. I.	°C.	Hrs.	%	%	%	
3.0	20	116	8	16.90	—	13.25	—
1.4	15	115	5	15.99	12.26	9.43	76.64
2.3	15	115	5	15.99	14.22	13.92	91.21
3.9	15	115	5	15.99	14.01	13.47	89.07
1.3	10	111	15	15.71	15.40	13.04	98.03
2.8	10	111	15	15.71	15.49	15.47	98.60
3.8	10	111	15	15.99	14.62	—	93.78

analysis of sorghum extracts.

Phytic acid was isolated from sorghum grain as barium phytate according to the procedure of Fiedler and Kärten (11). The procedure consisted of extracting phytic acid with 2 per cent HCl and adding barium chloride and sodium acetate to the extract to cause precipitation of barium phytate. The precipitate was dissolved in HCl and the precipitation was repeated several times to remove impurities. The final product was crystalline in nature. Analysis of the barium salt so obtained showed it to contain 14.96 per cent phytin phosphorus, 24.00 per cent inositol, and no inorganic phosphate. The inositol-phosphorus ratio was 0.989, indicating that the phytic acid of sorghum grain is inositol hexaphosphate.

The phytin inositol content of a few samples of sorghum grain was determined. The iron phytate which was precipitated from the sorghum extracts was transformed into sodium phytate by addition of NaOH and filtration to remove ferric hydroxide. This was necessary in order to eliminate the interference of iron in the subsequent oxidation of inositol.

The percentage of phytin inositol in the whole grain ranged from 0.30 to 0.37 per cent (Table 4). The average ratio of inositol to phosphorus was 0.97, which is in good agreement with 0.989 obtained with the barium phytate described above. These ratios indicate that with the phytin phosphorus method employed, non-phytin phosphorus did not appear in the final precipitate. If non-phytin phosphorus had been present, the

Table 4. Inositol and Phytin Phosphorus content of whole grain

Sample	Phytin P	Inositol	Inositol/P
	%	%	
Midland 316	0.26	0.26	1.00
Midland 304	0.28	0.27	0.96
Westland 356	0.21	0.20	0.95
Westland 366	0.28	0.27	0.96

inositol-phosphorus ratios would have been appreciably lower.

The good agreement between the values obtained by the two methods shows that the phytin phosphorus determination is a reliable method. Since it is somewhat simpler than the inositol determination, it alone was employed for determining the phytin content of various fractions of sorghum grains.

Phytin Content of Sorghum Grains

The phytin phosphorus of seven varieties of sorghum grain and of their fractions was determined (Table 5). The whole grain ranged from 0.20 to 0.37 per cent, Western Blackhull containing the greatest amount and Cody the least. Most of the phytin phosphorus was in the germ, the values ranging from 0.9% per cent (#356 Cody) to 1.91 per cent (#290 Westland.) The bran was the next highest, containing an average of 0.32 per

Table 5. Distribution of Phytin Phosphorus and Phytic Acid in sorghum grains and their fractions

Sample	Fraction	Total Acid Soluble P	Phytin P	Phytin P in Total Acid Sol. P	Phytic Acid Equivalent
		%	%	%	%
Westland Milo 287-1	Whole Grain	0.35	0.25	72	0.95
	Germ	1.23	1.09	88	4.17
	Bran	0.45	0.35	78	1.36
	Grit	0.05	0.03	58	1.23
	Mill Fines	0.17	0.13	74	0.49
Westland Milo 290-2	Whole Grain	0.33	0.27	81	1.02
	Germ	2.19	1.91	87	7.32
	Bran	0.59	0.49	83	1.88
	Grit	0.05	0.02	48	0.09
	Mill Fines	0.13	0.09	64	0.33

Table 5. (Cont'd)

Sample	Fraction	Total Acid Soluble P	Phytin P	Phytin P in Total Acid Sol. P	Phytic Acid Equivalent
		%	%	%	%
Blackhull Kafir 253-2	Whole Grain	0.35	0.25	72	0.95
	Germ	1.04	1.77	96	6.80
	Bran	0.39	0.34	87	1.30
	Grit	0.10	0.08	85	0.31
	Mill Fines	0.19	0.17	89	0.66
Western Blackhull 255-1	Whole Grain	0.41	0.37	91	1.43
	Germ	1.98	1.81	91	6.93
	Bran	0.25	0.20	80	0.77
	Grit	0.08	0.04	53	0.16
	Mill Fines	0.14	0.14	69	0.39
Midland Milo 256-1	Whole Grain	0.43	0.33	78	1.28
	Germ	1.98	1.69	88	6.49
	Bran	0.46	0.39	86	1.51
	Grit	0.09	0.04	50	0.16
	Mill Fines	0.21	0.17	83	0.66

Table 5. (Concl.)

Sample	Fraction	Total Acid Soluble P	Phytin P	Phytin P in Total Acid Sol. P	Phytic Acid Equivalent
		%	%	%	%
Pink Valdar 421	Whole Grain	0.41	0.33	80	1.27
	Germ	1.27	1.03	81	3.95
	Bran	0.25	0.19	76	0.72
	Grit	0.26	0.18	70	0.69
	Mill Fines	0.40	0.31	78	1.20
Cody 358	Whole Grain	0.26	0.20	78	0.78
	Germ	0.64	0.54	84	2.06
	Bran	0.30	0.24	81	1.18
	Grit	0.11	0.07	64	0.26
	Mill Fines	0.53	0.47	89	1.81

cent. In some cases, as for Coly and Pink Kafir, the values for the germ appear low and those for the mill fines appear high. This presumably is due to variations in dry milling, which in turn depends partially on the nature of the grain. Apparently, larger amounts of germ appear in the mill fines with some samples.

It will be noted that much of the acid soluble phosphorus was in the form of phytin. The percentage of phytin phosphorus in the acid soluble phosphorus varied considerably, however. For the whole grain, it ranged from 72 to 91 per cent; for germ, 83-96 per cent; for bran, 76-87 per cent; for grits, 48-85 per cent; and for mill fines, 64-89 per cent.

From these data, it was concluded that the phytin phosphorus content of sorghum grains is similar to that of corn (Jones et al., 26), the principal commercial source of phytin at the present time. The acid soluble phosphorus of corn also is principally phytin phosphorus, about 80 per cent of it being in this form.

SUMMARY

The phytin content of sorghum grains and their fractions was determined by measuring phytin phosphorus, and in a few cases by determining phytin inositol also. Good agreement was obtained between the two methods. Phytin was isolated quantitatively as ferric phytate at room temperature from 0.6 per cent HCl extracts of sorghum grains by the addition of ferric chloride.

The ferric phytate so obtained was decomposed by oxidation with H_2SO_4 and HNO_3, and the phosphorus was determined by the molybdenum blue method. The iron did not interfere with color formation with this reagent.

Phytic acid of sorghum grain was isolated as barium phytate, and analysis of this compound for phosphorus and inositol showed the phytic acid to be inositol hexaphosphate.

Seven varieties of sorghum grain and their fractions were analyzed for their phytin content. The phytin phosphorus content of the whole grain ranged from 0.20 to 0.37 per cent. The germ contained the greatest amount, the values being 0.94 to 1.31 per cent. The bran contained about 0.32 per cent, while the grit contained about 0.06 per cent. Most of the acid soluble phosphorus of the sorghum grains was in the form of phytin.

ACKNOWLEDGMENTS

The author wishes to acknowledge with sincere gratitude the valuable aid and advice rendered by Dr. Howard L. Mitchell, his major professor, throughout this investigation. Appreciation is extended to Dr. Harold M. Barlam who suggested the problem and supervised a part of the work thereon.

He also would like to thank Dr. E.G. Heyne, Mr. Richard A. Hahn and members of the faculty for aids and suggestions during the course of this investigation.

LITERATURE CITED

(1) Association of Official Agricultural Chemists.
Official methods of analysis of the association of official agricultural chemists. Washington, D.C., 7th ed. 1950.

(2) Anderson, R.J.
Phytin and Phosphoric acid esters of inosite. N.Y. Agr. Expt. Sta., Tech. Bull. 19, 1912.

(3) _____.
Concerning inosite phosphoric acids. Tech. Bull. 79, 1920.

(4) Averill, E.P. and C.G. King.
The phytin content of foodstuffs. Amer. Chem. Soc. Jour. 48:724-28. 1926.

(5) Bailly, M.C.
Determination of inositol in medicinal calcium and magnesium inositol phosphate preparations. J. Pharm. Chim. 28:199-200. 1938.

(6) Beadle, G.W.
An inositolless mutant strain of neurospora and its use in bioassays. J. Biol. Chem. 156:683-89. 1944.

(7) Bertow, Edward and William Wells Walker.
Inactive inositol and other products from starch factory steep water. Ind. Eng. Chem. 30:300-3. 1938.

(8) Harley, E.B.
Determining phytin phosphorus. Ind. Eng. Chem. Anal. Ed. 16:389-91. 1944.

(9) Hastcott, E.V.
The isolation and identification of "Bios I." J. Phys. Chem. 32:1094-1111. 1928.

(10) Hassey, R.E.
Phytin phosphorus in wort and beer. J. Inst. Brewing. 36:319-26. 1930.

(11) Fiechter, F. and F.H. Kärtsen.
A simple method for determining inositol and phytinates as well as definite crystalline barium phytinate. Biochem. Z. 254:138-47. 1932.

(12) Fleury, P., G. Poirot and Mme. Jean Fievet.
Oxidation of inositol by periodic acid. Compt. Rend. 220:604-6. 1945.

(13) Fleury, P., G. Poirot and P. Fievet.
Oxidation of inositol by periodic acid. Ann. Pharm. Franc. 5:209-18. 1947.

(14) Fleury, P. and Aurel Negoulos.
Chemical determination of inositol. Compt. Rend. 227:691-3. 1948.

(15) Fleury, P.
The chemical hydrolysis of inositol hexaphosphoric acid. Compt. Rend. 221:416-8. 1945.

(16) Harris, R.S. and L.M. Mosher.
Estimation of phytin phosphorus. Ind. Eng. Chem. Anal. Ed. 6:320-1. 1934.

(17) Heggen, H.H.N. and J.F. Keith.
Determination of phytic acid by oxidation of the inositol with periodic acid. Pharm. Weekblad. 83:801-5. 1948.

(18) Neuberg, Wolfgang and Herman Stadler.
Über eine Titrationsmethode zur Bestimmung des Phytins. Biochem. Z. 64:422-37. 1914.

(19) Knowles, F. and J.E. Watkin.
The amounts and distribution of some phosphorus and nitrogen compounds in wheat during growth. J. Agr. Sci. 22:755-66. 1932.

(20) Lindenfeld, E.
Über die Gewinnung von Inositol aus inositphosphorsäuren Salzen. Biochem. Z. 272:284-9. 1934.

(21) Lopez, R. Camaros and L. Moreno.
Phytin in flours and dietetic products. Anales Bromatol (Madrid). 3:245-7. 1951.

(22) McCance R.A. and E.M. Widdowson.
Phytin in human nutrition. Biochem. J. 29:2049-9. 1935.

(23) Michel-Durand, E.
Separation and estimation of phytin. Bull. Soc. Chim. Biol. 20:413-22. 1939.

(24) Mioni, Vincenzo and Andrea Gandini.
Colorimetric determination of phytin phosphorus. Ann. Chim. Applicata. 37:154-62. 1947.

(25) Platt, B.S. and E.G. Glock.
Estimation of inositol in animal tissues. Biochem. J. 37:709-12. 1943.

(26) Pons, Walter A., Mack F. Stansburg, Jr. and Carroll L. Hoffpauir.
Determining phosphoric compounds in plant materials. A. Assoc. Official Agr. Chem. 36:2. 492-504. 1953.

(27) Rather, J.B.
The determination of phytin phosphorus in plant products. Amer. Chem. Soc. Jour. 39:2506-15. 1917.

(28) Wiazownicka, H.
Solubility of phosphorus compounds of wheat flour and ability of phytin to combine with protein substances therein. Bull. Intern. Acad. Polonaise. Classe Sci. Math. Nat. Ser. B. 1:107-22. 1933.

(29) Wrenshall, C.L. and W.J. Dyer.
Organic phosphorus in soils: II. The nature of the organic phosphorus compounds. A. Nucleic acid derivatives, B. Phytin. Soil Sci. 51:235-48. 1941.

(30) Zinnadze, G.H.
Colorimetric methods for the determination of phosphorus. Ind. Eng. Chem., Anal. Ed. 7:227-30. 1935.

(31) Young, L.
Determination of phytic acid. Biochem. J. 30:252-7. 1936.

THE PHYTIN CONTENT OF SORGHUM GRAINS AND THEIR FRACTIONS

by

I-CHENG WANG

B. S., National Chekiang University, China, 1944

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Chemistry

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1956

Phytin is commonly known as the mixed calcium and magnesium salt of inositol hexaphosphoric acid. It is widely distributed in the plant kingdom. It has been used as a scavenger in various industrial processes. The phosphorus portion of phytin probably functions as a reserve phosphorus supply, to be used during germination prior to the absorption of phosphorus from the soil, while the inositol portion is of biochemical interest, having been shown to be "bios 1," a substance necessary for normal reproduction of yeast cells.

As a part of an extensive research program concerned with sorghum grains, it was of interest to determine the content and distribution of phytin in these grains in order to evaluate their nutritive value and potential industrial utilization.

The phytin content of sorghum grains and their fractions was determined by measuring phytin phosphorus, and in a few cases by determining phytin inositol also. The methods of analysis were established by using calcium phytate isolated from corn. Phytic acid was precipitated quantitatively as ferric phytate at room temperature from 0.6 per cent solutions by addition of ferric chloride. The ferric phytate so obtained was decomposed by oxidation with H_2SO_4 and HNO_3, and the phosphorus was determined by the molybdenum blue method. The iron did not interfere with color formation with this reagent.

For the determination of inositol, it was also necessary to isolate phytin as ferric phytate. The ferric phytate was

transformed into sodium phytate by the addition of sodium hydroxide, and the phytate was hydrolyzed at pH 2.8 for 15 hours at a pressure of 10 pounds per square inch. The inositol liberated was estimated by oxidation with periodic acid.

The inositol-phosphorus ratio of calcium phytate from corn was 0.997, which compares favorably with the theoretical ratio of 0.968 for inositol hexaphosphoric acid. Phytic acid of sorghum grain was isolated as barium phytate, and analysis of this compound for phosphorus and inositol showed it to be inositol hexaphosphate also.

In estimating the phytin content of sorghum grain, phytic acid was extracted by soaking the sample with 2 per cent HCl for one hour, with frequent swirling. Phytic acid was precipitated from the extract as described above, and either phosphorus or inositol determinations were carried out on the precipitate.

Seven varieties of sorghum grains and their fractions were analyzed for their phytin content. The phytin phosphorus content of the whole grain ranged from 0.20 to 0.37 per cent. The germ contained the greatest amount, the values being 0.54 to 1.91 per cent. The bran contained about 0.32 per cent, while the grits contained about 0.06 per cent. Most of the acid soluble phosphorus of the sorghum grains was in the form of phytin.